

PACHYTENE CHROMOSOME MORPHOLOGY AND ITS BEARING ON INTERSPECIFIC AND INTERGENERIC RELATIONSHIPS OF COIX*

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The genus *Coix* is a member of the tribe Maydeae (family Gramineae) which comprises seven genera. Two of these, *Zea* and *Tripsacum*, are native to the New World, while the rest, *Coix*, *Chionachne*, *Trilobachne*, *Sclerachne* and *Polytoca*, are native to southeast Asia (Mangelsdorf and Reeves, 1939). The Maydeae is a taxonomic rather than a natural assemblage of genera (Chaganti, 1965), with monoecism as the feature in common to both the New World and Asiatic members. However, from time to time, the existence of possible phylogenetic relationships between the two geographically separated groups has been postulated (Anderson, 1945; W.C. Galinat, personal communication).

Coix is the largest of the five maydeaceous genera native to Asia. Its systematics is confusing (Venkateswarlu

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and Chaganti, 1973). At least three highly polymorphic species assemblages are recognized, namely *C. aquatica* Roxb., *C. Lachryma-Jobi* L., and *C. gigantea* Koen. *C. aquatica* and *C. Lachryma-Jobi* have chromosome numbers of $2n=10$ and 20 respectively, while in the case of *C. gigantea* populations with chromosome numbers of $2n=20$ and 40 have been reported (Venkateswarlu and Chaganti, 1973).

Comparative chromosome morphology, especially at the pachytene stage of meiosis, where the chromosomes are more extended than at mitotic metaphase and often present characteristic features, is a useful parameter in understanding interspecific and intergeneric relationships. In the case of Coix, such studies have been few (Venkateswarlu and Chaganti, 1973). We have studied and report here the pachytene chromosome morphology in populations of Coix representing the three species.

Materials and Methods

Samples of seeds from populations representing the three species were assembled from different regions of India. Some were collected in the field by the authors, while others were collected for them by colleagues in different parts of the country. In addition, two samples from Japan and one from Brazil (countries in which Coix is not native, but into which it has been introduced for use as a fodder, cereal or ornamental) were also obtained. The geographic origin and method of collection of all the seed material is listed in Table I. Plants were raised from these seeds in the experimental gardens of the Department of Botany of the Andhra University. Young inflorescences were fixed in 3:1 ethanol-acetic acid. After 24–48 hours of fixation, the fixative was replaced by 70% ethanol. The material was stored in this fluid at 4°C , until used for study.

Acetocarmine squash preparations were made of young anthers. Appropriate nuclei were photographed, and camera lucida drawings were made of them from temporary preparations. Chromosomes were measured from camera lucida drawings alone, and their lengths, which include the centromeric regions, are expressed in micron units. Numbers are assigned to chromosomes on the basis of decreasing order of length: thus chromosome 1 is the longest of the complement. Individual chromosomes are identified on the basis of their length, arm-ratio (length of long arm/length of short arm), and the pattern of distribution of heteropycnotic and eupycnotic region.

Observations

Coix aquatica Roxb. The chromosomes of this species are difficult to study at pachytene, because they are long and often intertwined. In addition, the chromosomes possess long heteropycnotic segments which often exhibit non-homologous association (Plate LV), thereby making it difficult to follow the chromosome from one end to the other. Deep staining heteropycnotic regions are present in the proximal regions of both arms. Additional heteropycnotic regions are present at other locations on the chromosome arms. The chromosome arms terminate in pronounced and dark staining chromomeres. Exact measurements of the heteropycnotic regions were not possible to obtain, because of variability in their extent (see Venkateswarlu and Chaganti, 1973). Camera lucida drawings of the five chromosomes are represented in Plate LVI, while the idiogram represented in Plate LX is based on mean values of length and arm ratio of five measurements for each chromosome (see Table II). The third longest chromosome of the complement has the nucleolus organizer in the long arm in a sub-terminal position (Plate LX).

TABLE I
Geographic origin and mode of acquisition of seed material of the various populations belonging to the three species of Coix, viz., *C. aquatica* Roxb., *C. Lachryma-Jobi* L., and *C. gigantea* Koen, used in this investigation.*

Species**	Geographic Origin	Mode of Acquisition	Accession or Population Number
<i>C. aquatica</i>	Madhya Pradesh, India	supplied by: Prof. S.K. Pande, Saugar University	C.A.-6
"	Orissa, India	supplied by: Dr. G.V. Chalam, National seeds Corporation	C.A. 0-1
"	Andhra Pradesh, India	collected by one of the authors (RSKC)	C.A. 8
<i>C. Lachryma-Jobi</i> var. <i>typica</i> Walt.	Assam, India	supplied by: Regional Botanist, Botanical Survey of India	C-4a
"	Assam, India	supplied by: Regional Botanist, Botanical Survey of India	C-4b
"	unknown	unknown	C-7
"	Anantagiri in the coastal mountain range of southeastern Peninsular India (Eastern Ghats)	collected by one of the authors (RSKC)	C-8
"	Coimbatore, India	supplied by: Principal, Agricultural College	C-15
"	Campus Wild. A colony of plants growing wild on the Andhra University Campus. Could have started as escapees from cultures in the University Botanic Gardens. Primary source unknown.	collected by the authors	—

<i>C. Lachryma-Jobi</i> var. <i>monilifer</i> Walt	Dehra Dun, India	supplied by: Regional Botanist, Botanical Survey of India	C-10a
"	Dehra, Dun, India	supplied by: Regional Botanist, Botanical Survey of India	C-10b
"	Japan	supplied by: Dr. Harada, Crop Science and Breed- ing Laboratory, Department of Agriculture, Saikyo University, Kyoto	C-11
<i>C. Lachryma-Jobi</i> var. <i>stenocarpa</i> Stapf	Assam, India	supplied by: Regional Botanist, Botanical Survey of India	C-2
"	Japan	supplied by: Dr. Harada, Crop Science and Breed- ing Laboratory, Department of Agriculture, Saikyo University, Kyoto	C-3
<i>C. Lachryma-Jobi</i> var. <i>ma-yuen</i> (Romanet) Stapf	Assam, India	supplied by: Dr. Rolla S. Rao, Botanical Survey of India	C-5
"	Brazil	supplied by: Dr. R.V. Schaaffhauser, St. Amaro, Sao Paulo	C-1
<i>C. Lachryma-Jobi</i> Morphologically in- termediate between vars. <i>monilifer</i> and <i>typica</i> .	Anantagiri, India	collected by one of the authars (PNR)	C-6
<i>C. gigantea</i>	Coastal mountain range of south- western Peninsular of India (Western Ghats)	supplied by: Dr. Rolla S. Rao, Botanical Survey of India	C.G. 1

* We are grateful to the various people listed in column 3 who supplied us with seed samples.

** Herbarium specimens of all the species and varieties listed above have been deposited in the Andhra University Herbarium.

Coix Lachryma-Jobi L. Chromosomes of all the populations examined, with the exception of one (C-8 in Table I), show differential staining into eu- and heteropycnotic regions as in the case of *C. aquatica*. Heteropycnotic segments are always present in the proximal regions of both arms. In this species also the chromosome arms terminate in deep staining chromomeres. In addition, terminal as well as intercalary knobs are present. The sizes and numbers of knobs vary in the different populations. Thus, some populations are devoid of knobs (e.g., population C-4b), while others possess them (e.g., eight knobs are present in population C-4a). Detailed studies were not performed of knob frequencies in the various populations. A complete analysis of the pachytene chromosome morphology was achieved in one population (C-8), in which the chromosomes do not exhibit differential staining (Plate LVII, a, b). An idiogram (Plate LX) was constructed on the basis of average value of up to five measurements of lengths and arm ratios for each chromosome (see Table II). The sixth longest chromosome of the complement has the nucleolus organizer in the long arm in a nearly terminal position (Plate LX). Plate LVIII illustrates the nucleolus organizing chromosome in a population (campus wild) that shows differential staining of chromosome regions.

C. gigantea Koen. Of this species, populations have been reported by several investigators (Darlington and Janaki Ammal, 1945; Nirodi, 1955; Venkateswarlu and Chaganti, 1973) with chromosome numbers of $2n=20$ and $2n=40$. The population that we studied (C.G. 1, which is native to the mountain range on the west coast of peninsular India called the Western Ghats) exhibited variation in chromosome numbers ranging from $2n=18$ to $2n=22$. One of the plants with 18 chromosomes (which at meiosis forms nine bivalents) exhibited well

PLATE LV



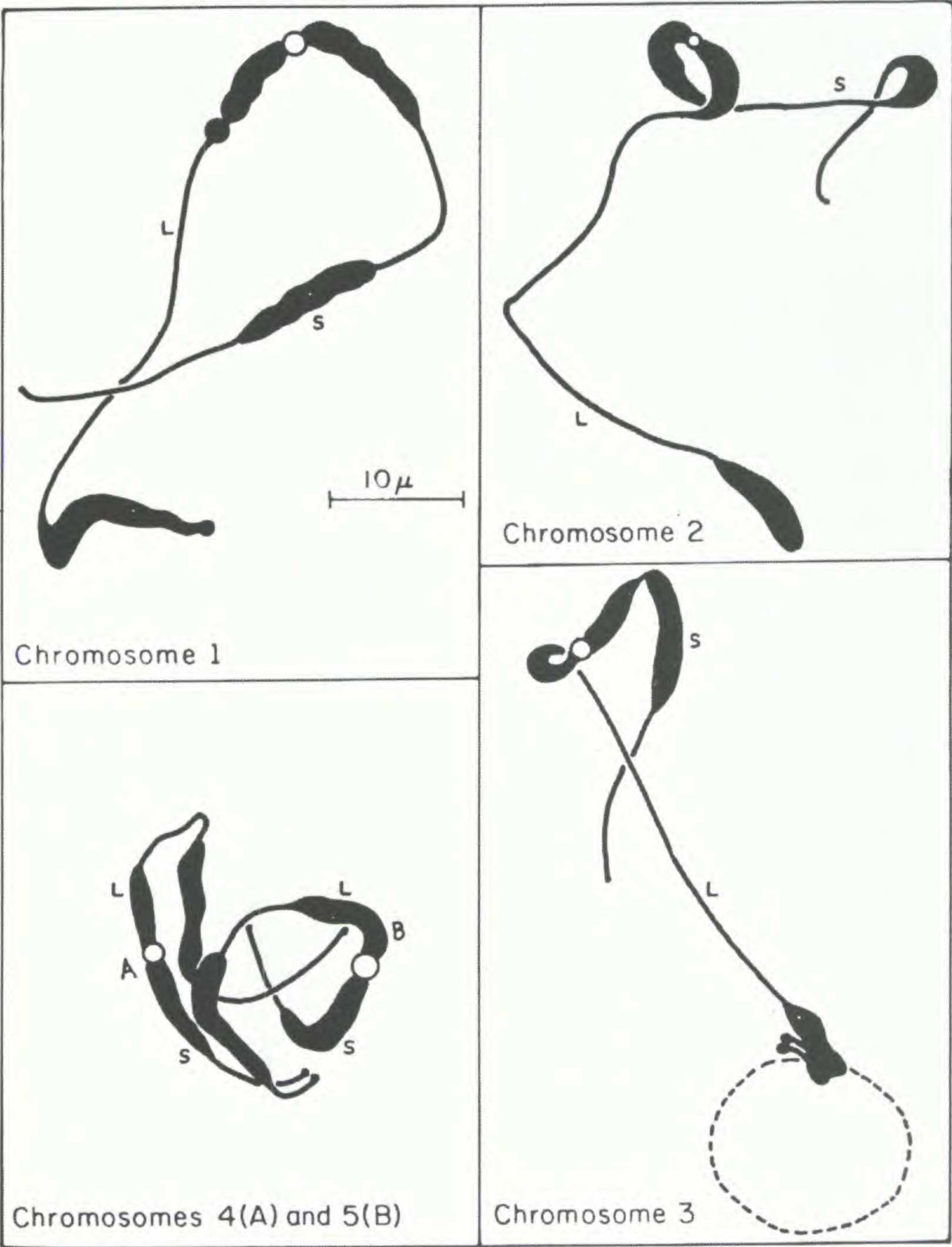
Portion of pachytene nucleus of *C. aquatica* showing non-homologous association of centromeres and heteropycnotic regions.

spread pachytene chromosomes (Plate LIX, a, b). All the chromosomes of this species, just as in the case of those of *C. aquatica* and *C. Lachryma-Jobi*, show differential staining into eu- and heteropycnotic regions, the latter being present in the proximal as well as interstitial regions of chromosome arms. Furthermore, the chromosome arms terminate in deep staining chromomeres. An idiogram constructed on the basis of mean values of at least ten measurements of lengths and calculation of arm ratios for each chromosome is presented in Plate LX (see Table II). The eighth longest chromosome of the complement has the nucleolus organizer situated almost terminally on the entirely heteropycnotic short arm (Plate LX).

Discussion

Variation in chromosome morphology and knob numbers in C. Lachryma-Jobi: The species of Coix are predominantly outbreeding. Four to six varieties have been recognized in *C. Lachryma-Jobi* based on morphological features of the fruit case (Mimeur, 1951; Bor, 1960). The varieties interbreed readily if grown together and allowed to open pollinate and their identity is lost within a few generations. Hence, the varietal differences are based on gene mutations, and the varieties arose through geographic isolation of populations or through selection by man. The variation observed at the population level in the chromosome phenotype: namely, the degree of heteropycnosis in chromosome arms and the sizes and numbers of knobs; can also be considered part of the same evolutionary processes that lead to varietal delineation. A detailed study of the variation of the chromosome phenotype has not yet been accomplished, but available information points to existence of a considerable amount of it. The fact that, in one of the populations

PLATE LVI

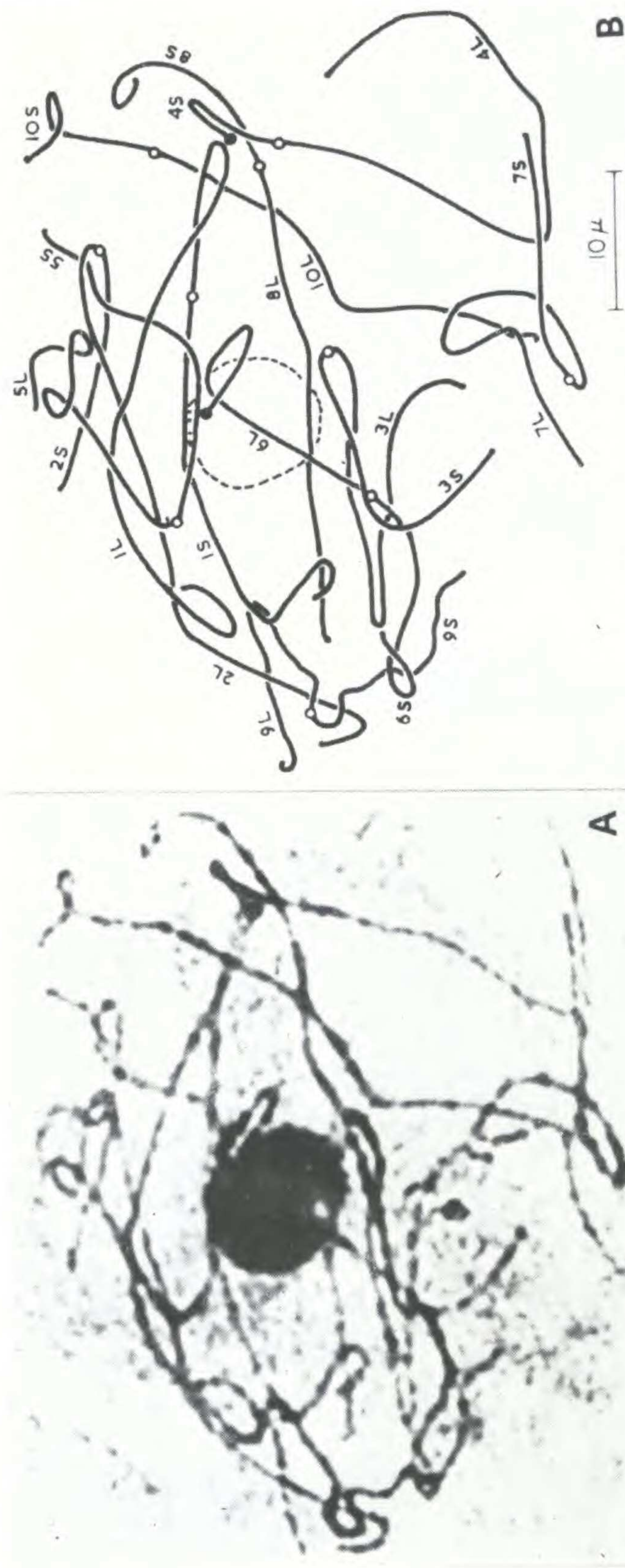


Camera lucida drawings of the five chromosomes of *C. aquatica* at pachytene.

(C-8), the chromosomes did not exhibit heteropycnosis might indicate that this feature is in some way controlled by the genotype. It is well known that several facets of chromosome form and behavior are under genotypic control (Rees, 1961; Chaganti, 1965). Knobs occur on the pachytene chromosomes of other members of Maydeae, e.g., *Zea* and *Tripsacum*. In these genera, numbers and positions at which knobs occur are characteristic features of populations. In *C. Lachryma-Jobi* var. *typica* collected at Coimbatore, a city in southwestern India, Nirodi (1955) observed five terminal and one interclary knobs. In a population (C-8) of the same variety collected at a different location (Anatagiri in the coastal mountain range in southern peninsular India called the Eastern Ghats), we found only two terminal knobs. In yet another population (C-4a) of the same variety originating from a different locality in India (Assam, a hilly province in eastern India), we found eight knobs. The relationship of knob variation to geographic distribution of the populations remains to be studied.

Non-homologous associations of centromeres and heteropycnotic regions in C. aquatica: In *C. aquatica*, at pachytene, non-homologous centromeres as well as non-homologous heteropycnotic regions are frequently associated (Plate LV). These associations, however, fall apart before diakinesis. The chromosomes of *C. Lachryma-Jobi* and *C. gigantea* exhibited none of these characteristics. The significance of such association is not known; however, it is likely that exchanges might take place in the associated regions and lead to reciprocal translocations as suggested by Venkateswarlu (1958). The genome of *C. aquatica* is characterized by a degree of instability; complex translocations are of frequent occurrence in natural populations (Venkateswarlu and Chaganti, 1973).

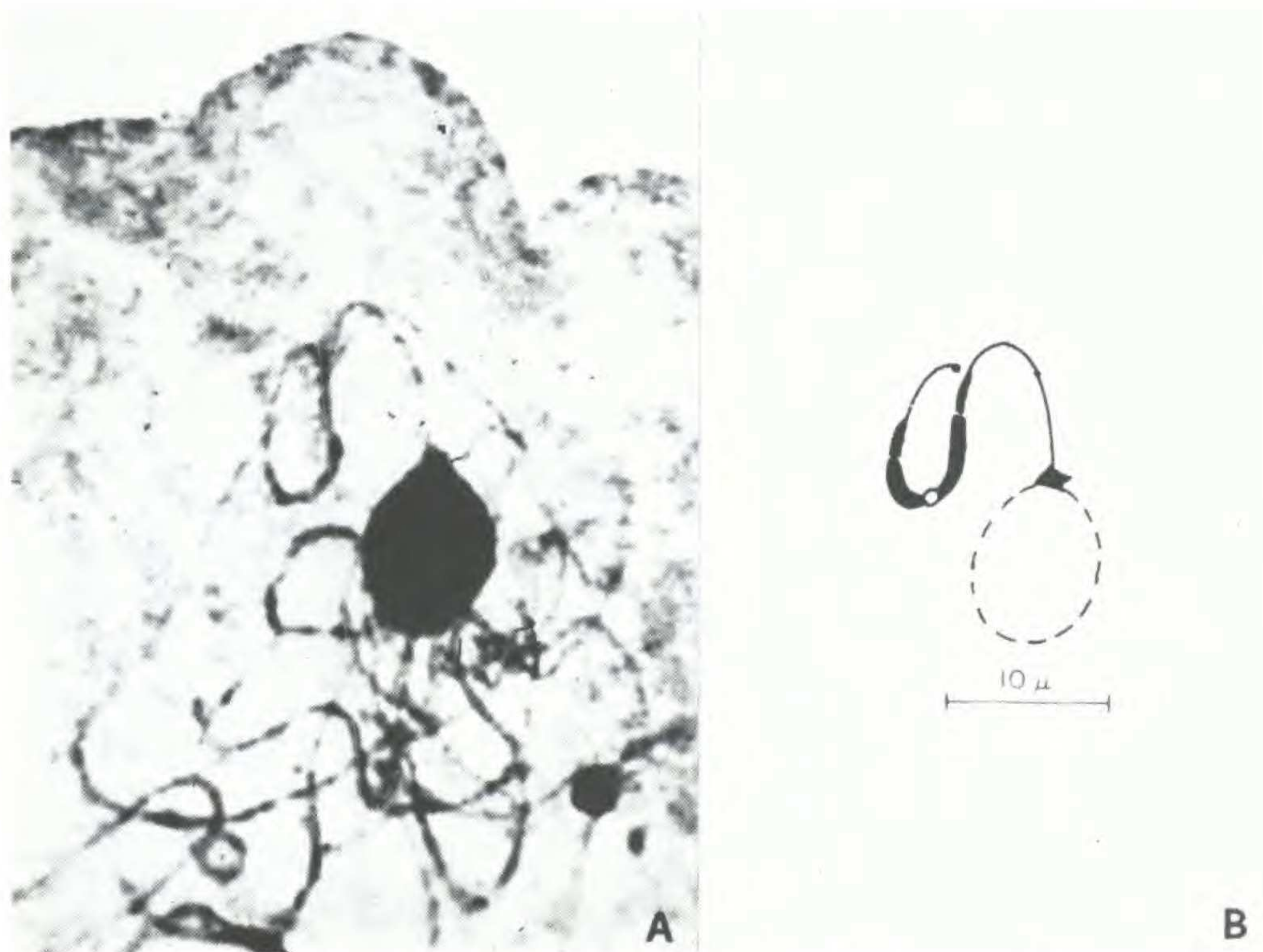
Comparison of the genomes of the three species: A com-



(a) The nucleus of a microsporocyte of *C. Lachryma-Jobi* var. *typica* (population C-8 in Table I) at pachytene. Differential staining of eu- and heteropycnotic regions is not evident. (b) A camera lucida drawing of the same nucleus. S=short arm; L=long arm; open circle=centromere; broken circle=nucleolus.

parison of the idiograms of the three species (Plate LX) reveals that, except for the nucleolus organizing chromosome, the chromosomes of the three species are quite dissimilar in their morphology. The length of each chromosome, its arm ratio, and the contribution its length made to the total length of the genome are given for the three species in Table II. *C. aquatica* has the longest chromosomes of the complement, with a genome which is 401.9μ long (mean chromosome length of 80.38μ). The genomes of *C. Lachryma-Jobi* and *C. gigantea* respectively are 582.0μ (mean chromosome length of 58.2μ) and 488.8μ (mean chromosome length of 54.31μ) long; thus *C. Lachryma-Jobi* and *C. gigantea* have genomes of approximately equal length. They are also shorter than that of *C. aquatica* by about 40%. The nucleolus organizing chromosomes of the three species, even though of widely differing lengths and arm-ratios, are similar; they are median to submedian, and the nucleolus organizer occupies a sub-terminal position. The dissimilar morphology of chromosomes between the presumably basic diploid *C. aquatica* and the derived polyploid species *C. Lachryma-Jobi* and *C. gigantea* must reflect, on the one hand, the effects of hybridity and chromosome doubling which presumably have been involved in the emergence of the species with higher chromosome numbers, and, on the other hand, the proneness of the genome of *C. aquatica* to re-patterning due to translocation (Venkateswarlu and Chaganti, 1973). Interspecific hybrids between *C. aquatica* and *C. Lachryma-Jobi*, *C. Lachryma-Jobi* and *C. gigantea*, and *C. aquatica* and *C. gigantea* are feasible, and the chromosomes in the F_1 hybrids exhibit intergenomic pairing (unpublished observations of Rao). Thus, at least one genome is common to all three species even though its presence cannot be detected by comparison of chromosome morphology alone.

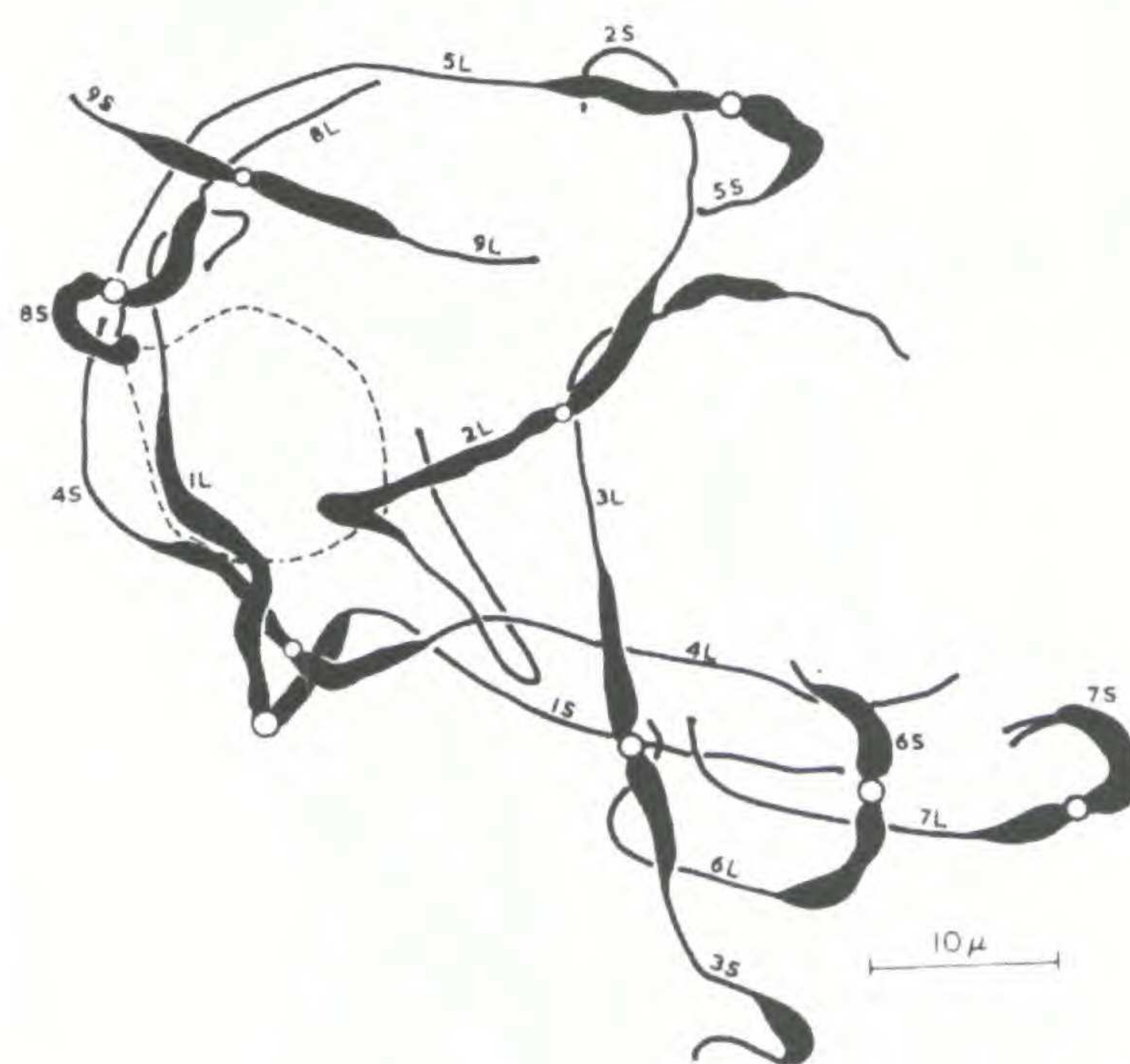
PLATE LVIII



(a) Portion of a pachytene nucleus of *C. Lachryma-Jobi* var. *typica* (population Campus wild) showing the nucleus organizer chromosome. This population exhibits differential staining of eu- and heteropachytic regions of the chromosomes which is evident in this cell. (b) A camera lucida drawing of the nucleolus organizing chromosome.

Comparison of the genomes of Coix, Sorghum and maize: The suggestion made by Anderson (1945) that maize originated in southeast Asia as an amphidiploid of a hybrid between species of *Coix* and *Sorghum* possessing a diploid chromosome number of 10, though rejected on a number of grounds (Mangelsdorf and Oliver, 1951; Chaganti, 1965), makes interesting a comparison of the morphology of the chromosomes of *C. aquatica* and species of *Sorghum* with a diploid chromosome number of 10 and those of maize. Pachytene chromosome morphology has been reported by other investigators in two species of *Sorghum* with a diploid chromosome number of 10: viz., *S. intrans* (Garber, 1947) and *S. purpureo-sericeum* (Reddi, 1958). The chromosomes of *S. intrans* are uniformly dark staining, and the centromeres are hard to locate in them. Three pairs are of one length; the remaining two are shorter and participate in nucleolus organization. The chromosomes of *S. purpureo-sericeum* are in the same size range as those of maize and possess dark staining chromomeres in the proximal regions of both arms, making the centromeres easy to locate. Neither species possess knobs. The genome of *C. aquatica*, at pachytene, is about 1.7 times longer than that of maize (data from this study compared to measurements of maize chromosomes at pachytene given in the publication of Rhoades, 1955), and the chromosomes themselves are quite dissimilar in morphology to those of maize. Chromosomes of *C. aquatica* show differentially stained eu- and heteropycnotic regions. They are also devoid of knobs. Maize chromosomes do not exhibit similar differential staining, and they possess knobs. Morphological comparisons, thus, do not reveal common features between the genomes of *C. aquatica*, *S. intrans*, and *S. purpureo-sericeum* on the one hand and maize on the other. This observation by itself, in the absence of

PLATE LIX



B

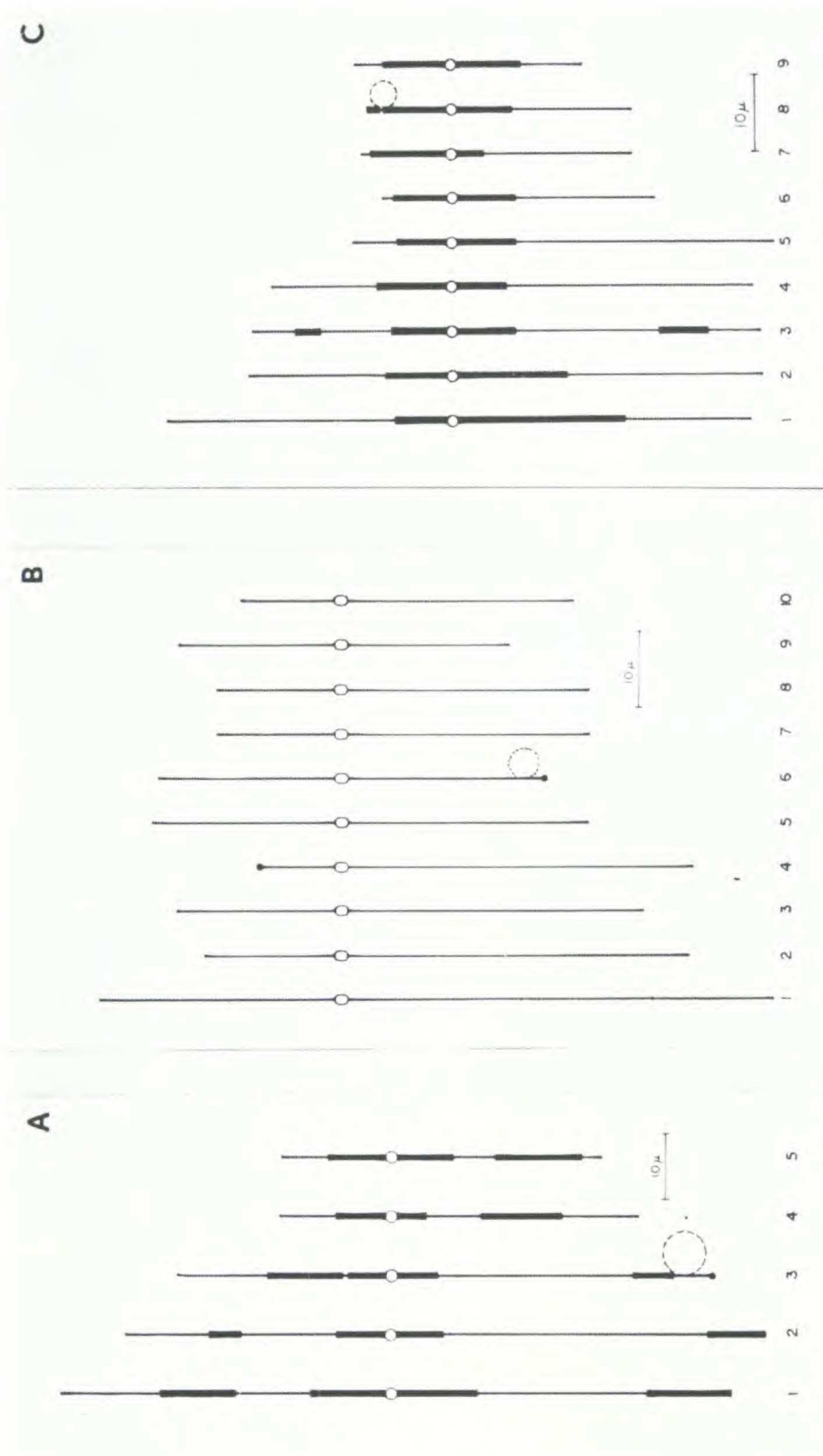
(a) The nucleus of a microsporocyte of *C. gigantea* at pachytene. Differential staining of eu- and heteropycnotic regions is clearly seen.
 (b) A camera lucida drawing of the same nucleus. S=short arm; L=long arm; open circle=centromere; broken circle=nucleolus.

genome analysis based on hybridization, cannot be considered evidence against possible existence of true phylogenetic relationships between these species. As discussed earlier, even in the case of the genus *Coix*, comparison of the chromosome morphology of the three species does not indicate the presence of the common genome demonstrated by intergenomic pairing of chromosomes in interspecific hybrids.

Chromosomes of *C. Lachryma-Jobi* and maize exhibit a few similarities. They fall in approximately the same length range at pachytene (data from this study compared to measurements of maize chromosomes given in the publication by Rhoades, 1955) as well as at diakinesis and metaphase I (Longley, 1941). Knobs, the sizes of which and positions on chromosomes vary in different populations, are present in both species. There is, however, no evidence based on genome analysis to indicate that these similarities reflect true phylogenetic relationships. The many attempts made at producing viable hybrids between maize and *Coix* have so far met with failure (Mangelsdorf and Reeves, 1939; Venkateswarlu, 1963).

Summary

Lengths, arm-ratios, and other morphological features of chromosomes at the pachytene stage of meiosis are reported for three species of *Coix*, namely, *C. aquatica* ($2n=10$), *C. Lachryma-Jobi* ($2n=20$), and *C. gigantea* ($2n=18$). The genome of *C. aquatica* is the longest of the three species and is about 40% longer than that of either of the other two species. When stained with acetocarmine the chromosomes of *C. aquatica* show conspicuous differential staining into eu- and heteropycnotic regions. They also exhibit non-homologous association of centromeres and heteropycnotic regions. *C. Lachryma-*



Idiograms of the pachytene chromosomes of *C. aquatica*, *C. Lachryma-Jobi*, and *G. gigantea* prepared on the basis of mean values of lengths and arm-ratios presented in Table II.

TABLE II
Chromosome lengths*, arm ratios, and percent relative contribution of each chromosome to the total genome length at pachytene in three species of Coix, viz., *C. aquatica* Roxb., *C. Lachryma-Jobi* L., and *C. gigantea*.

Chromosome No.	Species								
	<i>C. aquatica</i>			<i>C. Lachryma-Jobi</i>			<i>C. gigantea</i>		
	Length	Arm ratio	Percent contribution to the total genome length	Length	Arm ratio	Percent contribution to the total genome length	Length	Arm ratio	Percent contribution to the total genome length
1	107.7	1.04	26.80	92.0	1.81	15.81	80.0	1.05	16.37
2	101.3	1.44	25.20	66.0	2.62	11.34	71.2	1.54	14.57
3	84.6**	1.50	21.05	63.2	1.89	10.86	70.0	1.55	14.32
4	56.9	2.33	14.16	59.2	4.58	10.17	67.2	1.70	13.75
5	51.4	1.97	12.79	59.2	1.32	10.17	58.0	3.41	11.87
6				52.0**	1.10	8.93	38.0	3.09	7.77
7				50.0	2.08	8.59	37.6	2.07	7.69
8				50.0	2.08	8.59	36.0**	2.22	7.36
9				45.6	1.04	7.84	30.8	1.39	6.30
10				44.8	2.44	7.70			

* In microns. Mean value of up to five separate measurements in the case of *C. aquatica* and *C. Lachryma-Jobi* and ten measurements in the case of *C. gigantea*.

** Nucleolus organizing chromosome.

Jobi exhibits variation in chromosome morphology between populations in two features: (a) the extent of differential staining into eu- and heteropycnotic regions; and (b) the numbers and positions on chromosomes of knobs which are present on the chromosomes of this species. The chromosomes of *C. gigantea* show differential staining as in the case of *C. aquatica* and some populations of *C. Lachryma-Jobi*. But they are devoid of knobs. Comparative chromosome morphology does not reflect the presence of the common genome (or genomes) detected by interspecific hybridization.

Comparison of chromosome morphology does not show similarities between *C. aquatica*, species of Sorghum with a diploid chromosome number of 10, and maize; whereas some similarities are evident between the chromosomes of *C. Lachryma-Jobi* and those of maize. Data on genome analysis based on intergeneric hybridization between Coix, Sorghum, and maize are not available in order to interpret in genetic terms the morphological comparisons made in this study.

LITERATURE CITED

- Anderson, E. 1945. What is *Zea Mays*? A report of progress. Chron. Bot. 9: 88-92.
- Bor, N.L. 1960. Grasses of Burma, Ceylon, India and Pakistan. Pergamon Press, New York.
- Chaganti, R.S.K. 1965. Cytogenetic studies of Maize-Tripsacum hybrids and their derivatives. Bussey Inst. Harvard Univ. Publ., pp. 1-93.
- Darlington, C.D. and E.K. Janaki Ammal. 1945. Chromosome Atlas of Cultivated Plants. George Allen and Unwin, London.
- Garber, E.D. 1947. The pachytene chromosomes of *Sorghum intrans*. Journ. Hered. 38: 251-252.
- Longley, A.E. 1941. Chromosome morphology in maize and its relatives. Bot. Rev. 7: 263-289.
- Mangelsdorf, P.C. and D.L. Oliver. 1951. Whence came maize to Asia? Bot. Mus. Leaf. Harvard Univ. 14: 263-291.
- Mangelsdorf, P.C. and R.G. Reeves. 1939. The origin of Indian Corn and Its Relatives. Texas Agric. Expt. Sta. Bull. No. 574: 1-315.
- Mimeur, G. 1951. Systématique spécifique du genre *Coix* et systématique varietale de *Coix lacryma-jobi*. Morphologie de cette petite céréale et étude de sa plantule. Rev. Int. Bot. Appl. Agric. Trop. 31: 197-211.
- Nirodi, N. 1955. Studies on Asiatic relatives of maize. Ann. Mo. Bot. Gard. 42: 103-130.
- Rees, H. 1961. Genotypic control of chromosome form and behavior. Bot. Rev. 27: 288-318.
- Reddi, V.R. 1958. On the differentiation of A and B chromosomes of *Sorghum purpureo-sericeum* at pachytene. Jour. Ind. Bot. Soc. 37: 279-289.
- Rhoades, M.M. 1955. The cytogenetics of maize. In Corn and Corn Improvement. Ed. G.F. Sprague. Academic Press, New York, pp. 123-219.
- Venkateswarlu, J. 1958. Cytological observations on spontaneously occurring ring and chain formation in *Coix aquatica*. Jour. Ind. Bot. Soc. 37: 329-333.
- Venkateswarlu, J. 1963. Cytogenetic evolution in angiosperms-Maydeae. Mem. Indian Bot. Soc. No. 4: 65-73.
- Venkateswarlu, J. and R.S.K. Chaganti. 1973. Job's Tears. Tech. Bull. No. 44 Indian Council of Agricultural Research, New Delhi, pp. 1-54.